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Trace gas fluxes from managed grassland soil subject to multifactorial climate change manipulation

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ABSTRACT

Climate projections for the next decades expect a significant increase in air temperature and atmospheric CO_2 concentration, particularly in Alpine grassland. Most past experiments focused on individual climate changes parameters, such as warming (eT) and increase in atmospheric CO_2 (eCO_2). There is still little knowledge about these environmental changes, their magnitudes, and interactions on soil trace gas emissions and temperature sensitivity of associated microbial processes.

Therefore, we combined a multifactorial climate manipulation experiment with a laboratory incubation study. Intact soil cores were taken from the field site ("ClimGrass") following two years of treatment. To assess not only the effects of individual climate change factors (elevated CO_2 and elevated temperature) but also their combination on CO_2, N_2O, NO_x, and NH_3 a response surface model was applied after incubating soils in the lab. Also, temperature sensitivity of microbial processes involved in greenhouse gas production was determined.

In general, we found no interactions among treatments. However, the response surface regression suggests that a maximum of CO_2 emission occurred at the moderate CO_2 treatment (+150 ppm) while extreme CO_2 treatment (+300 ppm) showed similar CO_2 emissions as the control. NO_x emissions increased linearly with increasing temperature. Temperature sensitivity of associated microbial processes did not show a response to climate change treatments, likely due to a multitude of interacting soil and microbial processes.

This study highlights the importance of considering not only the combination of climate manipulations but even different steps of CO_2-enrichment and warming. We propose that more evident long-term trends are to be expected with sustained climate change manipulation treatments.

1. Introduction

Emissions of direct and indirect greenhouse gases (GHG) from terrestrial ecosystems have increased drastically over the last century. In particular, the atmospheric concentrations of carbon dioxide (CO_2) and nitrous oxide (N_2O), two important direct GHG, reached new historic highs in 2016 of 403.3 ppm and 328.9 ppb respectively, signifying an increase of 145% for CO_2 and 122% for N_2O since the pre-industrial period (IPCC, 2014; World Meteorological Organization, 2017). Indirect GHG such as nitrogen oxides (NO_x) and ammonia (NH_3) predominantly stem from the combustion of fossil fuels and production of fertilizer, respectively. However, trace gas production in soils should not be ignored as it has the capacity to increase tropospheric ozone (O_3) and acidify the soil through deposition of nitrate (NO_3^-). These processes can, in turn, lead to elevated N_2O emissions from soils, which have a global warming potential nearly 300 times that of CO_2 (Akiyama et al., 2004; Ferretti et al., 2017; Galloway et al., 2003; Smith et al., 1998).

Approximately 10–12% of the total global anthropogenic GHG emissions originate from the agricultural sector (Smith et al., 2008). The emission of GHG from grasslands to the atmosphere is of major concern since grasslands cover more than 20% of Europe (Eurostat, 2015) and non-CO_2 GHG emissions offset concurrent CO_2 uptake (Hörtnagl et al., 2018). There is considerable evidence that elevated CO_2 and warming affect trace gas fluxes by changing plant productivity, microbial activity and microclimate. Various single-factor manipulation

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experiments evaluating the effect of either warming or enhanced CO_2 on GHG emissions have been conducted in grasslands (Luo et al., 2006; Moser et al., 2018; Shaw et al., 2002; Zhou et al., 2008).

Elevated atmospheric CO_2 concentrations (eCO_2) can influence abiotic and biotic conditions in soil (van Groenigen et al., 2011), directly and positively affecting plant biomass production (Kammann et al., 2005; Morgan et al., 2004; Van Veen et al., 1991; Zak et al., 2000). Thus more organic C is available in soil (Van Veen et al., 1991) leading to higher microbial activity and respiration (Butterbach-Bahl and Dannenmann, 2011; Drissner et al., 2007; Wan et al., 2007) and ultimately increased CO_2 emissions. More C substrate provides more available energy for nitrifying and denitrifying bacteria and this, in turn, could boost soil N_2O and NO emissions (Hungate et al., 1997; van Groenigen et al., 2011; Van Veen et al., 1991). Along with an increase in atmospheric CO_2, enhanced water content has been reported (Dermody et al., 2007; Dijkstra et al., 2010; Nelson et al., 2004). This is mainly due to better water use efficiency in plants (Morgan et al., 2004), further supporting abiotic conditions that can increase soil N_2O emissions (Baggs et al., 2003; Kammann et al., 2007; van Groenigen et al., 2011).

Similarly, the effects of warming (elevated air temperature, eT) also influence multiple soil processes and therefore affect soil GHG emissions (Dieleman et al., 2012; Dijkstra et al., 2012; Mosier, 1998). If water and substrate are not limiting, warming will actively stimulate microbial activity and soil GHG emissions (Mosier, 1998; Pilegaard et al., 2006). However, since soil desiccation can strongly limit GHG emissions, more parameters besides temperature need to be considered when predicting warming effects of soil-atmosphere GHG exchange (Dijkstra et al., 2010; Selsted et al., 2012; Wan et al., 2007). For instance, CO_2 emissions from grassland soils are subject to seasonal and annual variability, which could be explained mainly by different temperature and precipitation patterns (Frank et al., 2002; Zhou et al., 2007). Although various studies have shown that warming treatments over the medium term (< 10 years) show higher CO_2 emissions (Rustad et al., 2001; Schindlbacher et al., 2011; Selsted et al., 2012), acclimation takes place on a long-term scale (Dieleman et al., 2012; Rustad, 2008). It is known that grassland ecosystems are particularly sensitive to increased temperatures, which can boost (gaseous) N-losses (Bijoor et al., 2008). Although temperature increase has been reported to decrease soil N_2O emissions (Jansen-Willems et al., 2016; Smith et al., 1998), the effects of warming on N_2O emissions are still unclear, since previous studies revealed highly variable rates (Dijkstra et al., 2012; Jansen-Willems et al., 2016). NH_3 emissions are indirectly affected by soil processes and are expected to increase with climate warming, but are as yet poorly understood (Flechard et al., 2013; Mosier, 1998).

Previous experiments mostly described difficulties in data interpretation when investigating the effect of a single climate parameter from field measurements, due to confounding parameters that co-vary or interact, such as N deposition, litterfall and N availability (Davidson et al., 2000; Pilegaard et al., 2006). These confounding parameters cannot provide unbiased estimates of trace gas emissions and related temperature sensitivity of microbial-derived GHG processes. Besides, complications can arise from the contribution of autotrophic soil respiration when measuring soil CO_2 emissions in the field (Schaufler et al., 2010).

Combined climate change treatments (eCO_2 and eT) caused inconsistent results in various studies (Niinistö et al., 2004; Selsted et al., 2012), as combined effects are still highly uncertain, regarding both the magnitude and the interactions (Yue et al., 2017). However, assuming that responses to environmental changes are not always linear, gradient approaches are needed but have rarely been performed (Kreyling et al., 2018).

A combined field-laboratory study was conducted to assess the influence of climate change on emissions of CO_2, N_2O, NO_x, and NH_3 and temperature sensitivity of microbial-derived GHG. To our knowledge, this is the first laboratory incubation study that considers multifactorial climate change treatments on direct GHG (CO_2 and N_2O) as well as indirect GHG (NO_x and NH_3) with samples obtained from an outdoor climate manipulation experiment.

Based on the complexity of the study our research questions were: R1) What are the effects of individual climate change factors (eCO_2 and eT) and their combination on trace gas emissions? R2) Do factorial climate change manipulations affect the temperature sensitivity of microbial processes involved in GHG emissions?

Our laboratory approach enables us to measure the legacy effects of field manipulation experiments on trace gas emissions and temperature sensitivity of microbial-derived GHG under controlled conditions. The inclusion of legacy effects will advance our understanding of the impacts of future climate change scenarios on soil trace gas emissions in grasslands.

2. Materials and methods

2.1. Site description

The soil cores for the incubation study were collected in 2016 from a pre-Alpine grassland that is part of the multifactorial climate manipulation experiment “ClimGrass”, established at the Agricultural Research and Education Centre (AREC) Raumberg-Gumpenstein, Austria (Herndl et al., 2011; Piepho et al., 2017). The experimental site is located at 47°29‘38“ N, 14°06‘03“ E at an altitude of 710 m a.s.L. (Richardson et al., 2012, 2018; Vivid Planet Software GmbH Internet Agentur und Webdesign Salzburg, n.d.), so is representative of the Alpine grassland region. In 2016 the mean air temperature was 9.1 °C, and the mean annual precipitation amounted to 1142.6 mm (data from the national ZAMG weather station; a climate diagram of the year 2016 is provided in the Supplementary Material, Fig. S.1). The soil is classified as Cambisol with loamy texture consisting of 44.2% sand, 47.6% silt, and 8.3% clay with a C:N ratio of 12.6 (WRB, 2015). The grassland was established in 2007 and can be classified as nutrient-rich meadow that primarily consists of tall oat-grass (*Arrhenatherum elatius* L.), Kentucky bluegrass (*Poa pratensis* L.), meadow fescue (*Festuca pratensis* L.) and orchard grass (*Dactylis glomerata* L.). During the growing season, from the beginning of April to the end of October, the grassland is harvested three times and mineral fertilizer is applied in three batches. This results in a total load of 90 kg N ha^{-1} y^{-1}, 65 kg P ha^{-1} y^{-1} and 170 kg K ha^{-1} y^{-1}. The field experiment is designed by a response surface approach (Piepho et al. 2017), based on a range of combinations of three levels of air temperature (ambient, +1.5 °C and +3 °C) and atmospheric CO_2 (ambient, +150 ppm and 300 ppm) to test explicitly non-linear and non-additive effects of warming and eCO_2 (Table 1). C0T0 (combination of ambient temperature and ambient atmospheric CO_2) indicate ambient conditions in the field and is handled as a control. Moderate treatments include C0T1 (increase in temperature by 1.5 °C), C1T0 (increase in CO_2 by 150 ppm) and C1T1 (combination of an increase in temperature by 1.5 °C and increase in CO_2 by 150 ppm). The extreme treatments are C0T2 (increase in temperature by 3 °C), C2T0 (increase in CO_2 by 300 ppm) and C2T2 (combination of an increase in temperature by 3 °C and increase in CO_2 by 300 ppm) (Table 1). The latter treatment (C2T2) represents the most

Table 1
A 3 × 3 response surface design with treatment-codes (C = atmospheric CO_2, T = Temperature) for a total of 27 plots, the number of replicates is given in parenthesis.

	eCO_2 (ppm)		
eT (C°)	0	150	300
0	C0T0 (7)	C1T0 (3)	C2T0 (3)
1.5	C0T1 (3)	C1T1 (2)	
3	C0T2 (3)		C2T2 (6)

likely future climate scenario that is predicted for the alpine region by the end of this century (Gobiet et al., 2014). The treatments were completely randomized. More details about the experimental set-up are provided in the Supplementary Material as well as in Pötsch and Herndl (2014) and Piepho et al. (2017). Briefly, warming treatments were realized with infrared heaters as described by Kimball (2011), and for the eCO_2 treatment, an adapted miniFACE system was installed according to Miglietta et al. (2001). The warming operation and CO_2-fumigation started in 2014. Warming treatments were performed full-time all year and only stopped when the snow cover exceeded a height of 10 cm. CO_2-fumigation was only done from the beginning of April to end of November and during the day when radiation energy was higher than 50 W m^{-2}.

2.2. Laboratory incubation and analysis

In this study 27 out of a total of 54 plots (size of 4 × 4 m each) of the experimental site were selected, since the remaining plots were used for other experiments (see Supplementary Material). The selected set of 27 plots enables the application of the response surface approach. Two intact soil cores per plot were taken in autumn 2016 before the last fertilization using a 7 cm high stainless steel cylinder with a 7.3 cm inner diameter. Intact soil cores were used to avoid disturbance effects caused by sieving (Brumme et al., 1999). After sampling the soil, cores were cooled, brought to the laboratory and stored at 4 °C until further treatment. The aboveground biomass was removed, and soil cores were fertilized with a 20 mL solution representing an equivalent of 30 kg N ha^{-1} in the form of ammonium nitrate. Two different incubators were used, one to measure CO_2, N_2O and NO_x emissions (Schaufler et al., 2010; Schindlbacher et al., 2004) and the other to determine NH_3 emissions (Ferretti et al., 2017; Haller, 2015). The number of soil cores exceeded the number of jars that could be placed into an incubator at the same time; therefore soil cores had to be processed in batches. Batches were chosen randomly, without any particular order, and were taken into account for statistical analysis (more details are provided below in Section 2.3). Between the measurements soil cores were stored at 4 °C to diminish effects on field treatments.

NH_3 emissions were measured by incubation for 12 h in an in-house built, fully automated device for soil emission measurements of reduced nitrogen, connected to a Picarro G2103 NH_3/H_2O CRDS analyzer (Ferretti et al., 2017; Haller, 2015). Measurements took place in an air-conditioned laboratory set to room temperature (22 °C), and test chambers were put into a polystyrene box to minimize the influence of diurnal temperature fluctuations. Six test chambers (ø 7.8 cm and 7.8 cm height) and tubes of the incubation device were built of Polytetrafluoroethylene (PTFE) to avoid NH_3 retention. Inlets of the test chambers were closed with rubber septa and outlets were locked by valves on the PFTE tubes, dried compressed air was used as carrier gas with a constant flow of 1 L min^{-1}. Five test chambers were filled with intact soil cores; one remained empty and served as a reference chamber to measure NH_3 background concentration. Emissions of NH_3 were calculated as described in Ferretti et al. (2017) and represented as cumulative emissions.

Before CO_2 and NO_x emissions were determined at different temperature levels (5, 10, 15 and 20 °C), water content was re-equilibrated at 4 °C for three days using distilled water. The equilibration at 4 °C was chosen due to the fact that (i) microbial processes responsible for trace gas production are predominantly driven by moisture and temperature (Schaufler et al., 2010), (ii) samples stored at 4 °C are more similar to fresh soil samples when measuring enzyme activity, fungal markers as well as soil respiration (Lee et al., 2007), and (iii) measuring CO_2 and NO_x emissions at different temperature levels (5, 10, 15 and 20 °C) started with the lowest temperature (5 °C) and sequentially increased at 5 °C intervals; the re-equilibration temperature of 4 °C is close to the starting temperature of 5 °C. Re-equilibration for a period of 3 days seemed sufficient to avoid trace gas emission measurement of the re-wetting effect.

The measuring system for CO_2 and NO_x determination was fully automated, as described in Schindlbacher et al. (2004). This system was based on an open flow method and consisted of a temperature-controlled incubator. The incubator was equipped with 24 adapted Kilner jars, serving as test chambers and one was used as blank to measure gas background in laboratory air. The concentration of CO_2 was measured using a WMA-4 infrared gas analyzer (PP-Systems, Hitchin UK). The NO_x concentration was determined using a chemiluminescence analyzer (Horiba Apna-360, Kyoto Japan). CO_2 and NO_x emissions were calculated according to Schindlbacher et al. (2004). Since the average air temperature during growing season 2016 was 15.3 °C, cumulative emissions of CO_2 and NO_x (cum CO_2 and cumNO_x, respectively) were calculated at 20 °C over a period of 18 h. This temperature was selected because alpine regions are sensitive ecosystems and it is expected that mean summer temperature will increase by 3–5 °C in the next 100 years. Even greater temperature increases are expected in Europe's temperate regions (Koch et al., 2007). The actual temperature sensitivity for CO_2 (Q_{10}) was calculated according to Winkler et al. (1996). Besides, the data were fitted to the generalized exponential function presented by Lloyd and Taylor (1994) (for more details see below in Section 2.3).

Nitrous oxide emissions were determined directly after CO_2 and NO_x measurements based on a closed chamber method as described in Schindlbacher et al. (2004) and Schaufler et al. (2010). Before N_2O measurements, jars were flushed with ambient air to avoid gas accumulation at the bottom. Inlets of the test chambers were closed with rubber septa, and the outlets were locked by valves on the PFTE tubes. Nitrous oxide gas samples were taken manually with a syringe at 20-minute intervals (4 samples per hour) at each of the temperature levels described above. Samples were injected in pre-evacuated 10 ml glass vials (Agilent Technologies) and then analyzed with gas chromatography using a ^{63}Ni electron capture detector (ECD). With the change in headspace concentration over time, the N_2O emissions were calculated by linear regression ($R^2 < 0.8$) according to Díaz-Pinés et al. (2014). Emission values of ≤ -50 µg N_2O-N m^{-2} h^{-1} were excluded in further calculations and remaining values represented as mean N_2O emissions. These negative fluxes were only partly observed at the low incubation temperatures, where microbial processes are expected to be low. Especially lower fluxes that occur at a lower temperature may show net uptake when measurements are close to detection limits (Chapuis-Lardy et al., 2007). The actual and fitted temperature sensitivity were calculated for N_2O following the same procedure as it was for CO_2 (Lloyd and Taylor, 1994; Winkler et al., 1996) (for more details see below in Section 2.3).

It is important to consider that the long time lag between fertilizer application and trace gas measurements and consecutive incubations may have effects on substrate availability since the longer the incubation lasts, the more substrate will be used and less will be available. However, the potential bias is assumed to be equal for all cores for comparison of results (Schaufler et al., 2010).

After gas emission measurements, the soil of intact cores was sieved (< 2 mm), separated from roots and gravel, and homogenized for determination of different soil characteristics. Soil moisture content was determined by oven drying for 24 h at 105 °C and expressed as water-filled pore space (WFPS). Roots and stones were washed and air dried, and root mass (mg g^{-1} dry soil dry matter) was calculated. An amount of 2.5 g soil was extracted with 2 M KCl in a 1:10 w/v ratio as described in Brandstätter et al. (2013). For microbial carbon (C_{mic}) and nitrogen (N_{mic}), chloroform fumigation extraction (CFE) was applied according to (Schinner et al., 1996). CFÉs and non-fumigated soil extractions were measured with an automated TOC/TN analyzer (TOC-V CPHE200V), linked with a TN-unit (TNM-1 220 V, Shimadzu Corporation, Kyoto, Japan). These data allowed the calculation of dissolved organic nitrogen (DON), extractable total nitrogen (ETN) and extractable organic carbon (EOC). Further, the ammonium (NH_4^+) concentration was

Table 2
Least square means ± SE for cumulative CO_2, NO_x and NH_3 fluxes as well as mean N_2O fluxes.

		eCO_2 (ppm)					eCO_2 (ppm)		
	eT (°C)	0	150	300		eT (°C)	0	150	300
Cumulative CO_2 ($g\ m^{-2}$)	0	23.12 ± 0.2	42.61 ± 0.3	32.13 ± 0.3	Mean N_2O-N ($mg\ m^{-2}h^{-1}$)	0	1.43 ± 0.4	0.40 ± 0.6	0.90 ± 0.6
	1.5	40.87 ± 0.3	46.38 ± 0.3			1.5	0.25 ± 0.6	0.71 ± 0.8	
	3	25.94 ± 0.3		25.04 ± 0.2		3	0.39 ± 0.6		0.56 ± 0.4
Cumulative NO_x-N ($mg\ m^{-2}$)	0	1.45 ± 0.1	1.46 ± 0.1	1.30 ± 0.1	Cumulative NH_3-N ($\mu g\ m^{-2}$)	0	3019.96 ± 0.2	3402.96 ± 0.3	2589.45 ± 0.3
	1.5	1.92 ± 0.1	1.46 ± 0.2			1.5	2762.80 ± 0.3	3948.93 ± 0.4	
	3	1.56 ± 0.1		1.81 ± 0.1		3	1888.24 ± 0.3		3515.02 ± 0.2

[#] Least square means ± SE are not significantly different at the 5% level of significance.

measured by Berthelot reaction according to Schinner et al. (1996), and the nitrate (NO_3^-) concentration was determined as described in Hood-Nowotny et al. (2010) in transparent microplates on a spectrophotometer (PerkinElmer® type 2300 EnSpire™). Additionally, a subset of soil cores was used to determine soil texture via particle size distribution (ÖNORM L 1061-2 KG).

2.3. Calculations and statistical analysis

All emission calculations, as well as calculations for basic soil parameters, were carried out in Excel 2013 (Version 15.0.4997.1000). To study the effect of single climate change factors and combinations of these on CO_2, N_2O, NO_x and NH_3 emissions, a linear mixed model was fitted with SAS (Version 9.4). All flux data were log-transformed, except data for N_2O at different temperature levels which were square root transformed to stabilize the variance. The experiment had two different phases, i.e., a field phase and a laboratory phase, and for statistical analysis a linear mixed model was fitted including design effects for both phases (Brien et al., 2011). For the field phase, which was completely randomized, random effects were fitted for plots. The random effects for the two soil cores per plot were modeled by the residual error term, and for the laboratory phase, a random effect was fitted for batches.

Two types of analyses for the two treatment factors were performed. In the first analysis, eCO_2 and eT were implemented as qualitative factors with the two main effects and two-way interaction. In the second analysis a second-order response surface model was fitted (Piepho et al., 2017). These analyses were performed for those characteristics for which a single measurement per experimental unit was taken in the lab (main soil biotic and abiotic parameters, cumulative CO_2, mean N_2O emissions, cumulative- NO_x and NH_3 emissions). For characteristics measured repeatedly at increasing incubation temperatures (5, 10, 15, 20 and 25 °C), first-order autoregressive model [AR(1)] (Verbeke, 1997) variance-covariance structures were fitted for the random design effects, i.e. plots, batches and soil cores in order to account for serial correlation. Besides, fixed effects were added for temperature and interactions with eCO_2 and eT. In the case of response surface regression, the temperature was added as another quantitative regressor variable. Generally, in response surface regression, a second-degree model was fitted initially including a check for lack-of-fit. If the lack-of-fit was not significant, which was generally the case, subsequently the second-order terms were checked, removing non-significant ones. After this, linear terms were inspected in cases where the corresponding quadratic term was not significant (Piepho and Edmondson, 2018). Actual Q_{10} values were calculated as the factor by which measured CO_2 or N_2O emission rate increased for every 10 °C step,

$$Q_{10} = \frac{R_{T+10}}{R_T}$$

with R_T and R_{T+10} representing the CO_2 or N_2O emission rates at temperature T and T + 10. For the LTQ_{10} analysis, first data as well as the model were log-transformed, to estimate model parameter and predicted values of $\log(R_T)$

$$\log(RT) = \log(R1) + \frac{a}{b + T}$$

where $R1 > 0$, $a < 0$, $b < 273.15$. Subsequently, predicted values were back-transformed, and Q_{10} was calculated.

Pearson correlations (ρ) were estimated in SigmaPlot 12.0 between trace gas emissions and measured soil parameters across all treatments. However, for the correlation of NO_x and N_2O means of soil gas emissions were used.

3. Results

3.1. Cumulative CO_2 emissions

Soil respiration tended to increase relative to the controls for all field treatments at a laboratory incubation temperature of 20 °C. Highest increases of 100.6%, 76.8%, and 84.3% compared to the ambient situation (C0T0) were detected for the C1T1, C0T1, and C1T0 treatments, respectively (Table 2). The response of cumulative CO_2 emissions ($cumCO_2$ in $g\ m^{-2}$) to treatments is presented in the Contour plot (Fig. 1) which shows that the maximum of CO_2 emission occurred at moderate eCO_2 ($F = 3.86$, $p = 0.0550$) and decreased again at extreme scales. Moreover, $cumCO_2$ correlated positively with root biomass (ρ = 0.277), NH_4^+ (ρ = 0.285) and DON (ρ = 0.434) (Table 3).

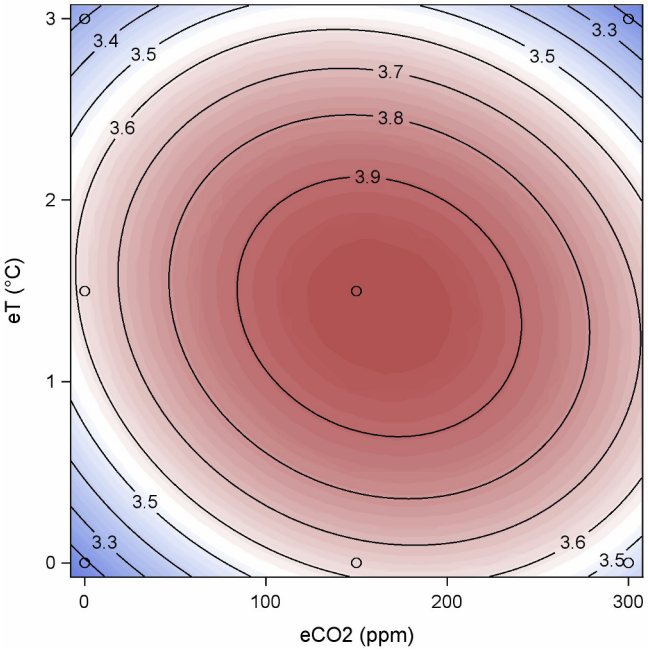

Fig. 1. Contour plot for cumulative CO_2 fluxes (in $g\ m^{-2}$) along with a gradient of increased temperature (y-axes) and elevated atmospheric CO_2 (x-axes) for soil cores incubated at 20 °C for 18 h.

Table 3
Pearson correlations between cumulative CO_2, NO_x and NH_3 and mean N_2O gas fluxes and biotic/abiotic soil parameters.

	Pearson correlation (ρ)			
	$cumCO_2$	Mean N_2O	$cumNO_x$	$cumNH_3$
Root biomass	**0.277**	**−0.313**	−0.0398	−0.136
WFPS	**−0.434**	**0.503**	−0.144	0.126
EOC	0.344	−0.0901	0.216	−0.0585
ETN	0.135	0.0094	**0.376**	−0.0665
C_{mic}	0.221	−0.0585	0.0452	−0.0142
N_{mic}	−0.119	0.146	0.0456	−0.048
NH_4^+	**0.285**	−0.0822	0.167	0.0112
NO_3^-	−0.216	0.128	**0.349**	−0.0934
DON	**0.434**	−0.129	0.0285	0.00156
C/N	−0.071	0.0784	−0.0418	−0.226

3.2. Mean N_2O emissions

Mean N_2O emissions (in mg m^{-2} h^{-1}) tended to decline with climate change treatments. C0T1, C0T2, and C1T0 showed the lowest mean N_2O emissions (0.25, 0.39 and 0.40 mg m^{-2} h^{-1}, respectively), but differences are statistically not significant (Table 2). However, mean N_2O emissions correlated positively with WFPS (ρ = 0.503) (Table 3).

3.3. Cumulative NO_x emissions

C0T1 showed highest NO_x emissions (+32% compared to C0T0), in contrast C0T2 reacted less (+7.6% compared to C0T0) (Table 2). The $cumNO_x$ responded significantly to the temperature in the response surface analysis (F = 4.31, p = 0.0429), as well as in the first order-model (Contour plot) it is shown the positive effect of temperature on $cumNO_x$ emissions (Fig. 2). Cumulative NO_x emissions ($cumNO_x$ in mg m^{-2}) correlated positively with ETN (ρ = 0.376), and with NO_3^- (ρ = 0.349) (Fig. 3). A negative correlation (ρ = −0.764) occurred between mean N_2O and mean $cumNO_x$ emissions.

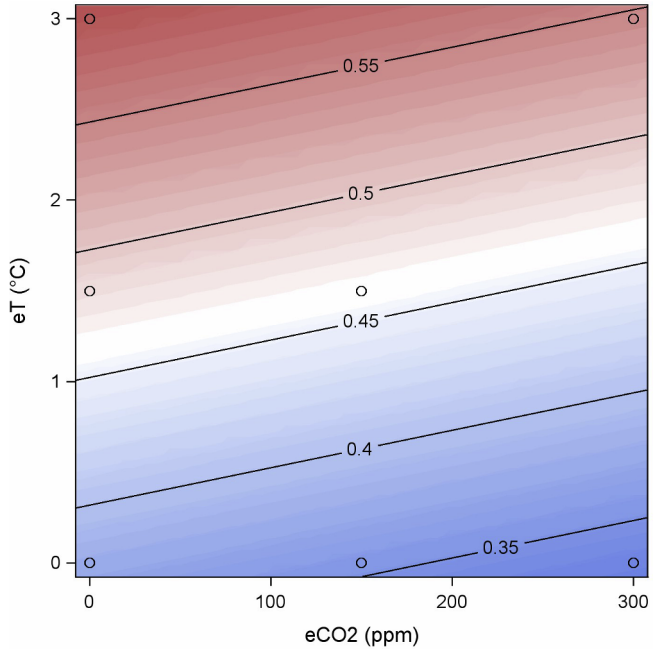

Fig. 2. Contour Plot for cumulative NO_x fluxes (in mg m^{-2}) along with a gradient of increased temperature (y-axes) and elevated atmospheric CO_2 (x-axes) for soil cores incubated at 20 °C for 18 h.

3.4. Cumulative NH_3 emissions

Cumulative NH_3 emissions ($cumNH_3$ in µg m^{-2}) tended to decrease at the C0T1, C0T2 and C2T2 treatments (Table 2).

3.5. Temperature sensitivity (Q_{10})

3.5.1. CO_2

Soil CO_2 emission increased with rising incubation temperature for all field treatments (Fig. 3a and 3b). The included incubation temperature considered as a repeated measurement factor was significantly affecting the CO_2 emissions (F = 39.48, p = < 0.0001) (Table 4).

Actual Q_{10} (Fig. 3c and 3d), as well as LT Q_{10} (Fig. 3e and 3f), decreased with increasing temperature at moderate and extreme treatments.

3.5.2. N_2O

Mean N_2O emissions increased with successively rising incubation temperature. The impact of the included incubation temperature was highly significant (F = 54.16, p = < 0.0001) (Table 4). Additionally, the N_2O emissions showed a significant response to the main temperature effect (F = 3.97, p = 0.0214) as shown in Table 4. In the response surface regression N_2O decreased significantly with field phase temperature (F = 6.30, p = 0.0196) and increased with laboratory phase incubation temperature (F = 320.76, p = < 0.0001). Thus, N_2O emissions increased linearly with rising incubation temperature, and there was less N_2O production in warmed treatments compared to the control (Fig. 4).

The actual Q_{10} (Fig. 5c and d) decreased only with increasing incubation temperature in the treatments C0T0, C1T0, and C2T2, whereas in C1T1 and C2T2 it increased with temperature. In comparison with the actual Q_{10}, the LTQ_{10} values of N_2O (Fig. 5e and f) decreased only slightly with increasing temperature.

4. Discussion

The experimental design of our study allowed us to assess CO_2, N_2O, NO_x and NH_3 gas measurements from a laboratory incubation study with samples obtained from a field manipulation experiment that has been subjected to single and combined climate change factors in a response surface approach for a period of 2 years. Measurements of those gaseous losses are still scarce in the literature (Larsen et al., 2011), but the awareness of their potential interaction is increasing (Brown et al., 2012; Yue et al., 2017).

4.1. Soil CO_2 emissions

In our study, all tested climate change treatments tended to stimulate cumulative CO_2 emissions ($cumCO_2$) compared to C0T0 (Table 2). Previous studies have found positive effects of elevated air temperature (eT) and elevated atmospheric CO_2 (eCO_2) on soil CO_2 emissions (Craine et al., 2001; Selsted et al., 2012; Wan et al., 2007).

Typically, eCO_2 is found to alter soil CO_2 emissions during the growing season (Craine et al., 2001). This is likely due to its positive effects on photosynthesis, above- and below-ground plant biomass production, subsequent greater root respiration and enhanced substrate availability for soil microorganisms leading to greater C losses by soil microbial respiration (Dieleman et al., 2012; Dusenge et al., 2018; Morgan et al., 2004; Zak et al., 2000). In our study, C supply was interrupted by taking the cores from the field. Therefore, our lab incubation results reflect indirect legacy effects from field manipulations as well as direct temperature effects during incubation.

Considering eT treatments, soil respiration was positive, though not significantly affected by temperature treatments in the field (Table 2). Literature also revealed stimulated soil respiration in the first years of climate warming experiments in different ecosystems (Dieleman et al.,

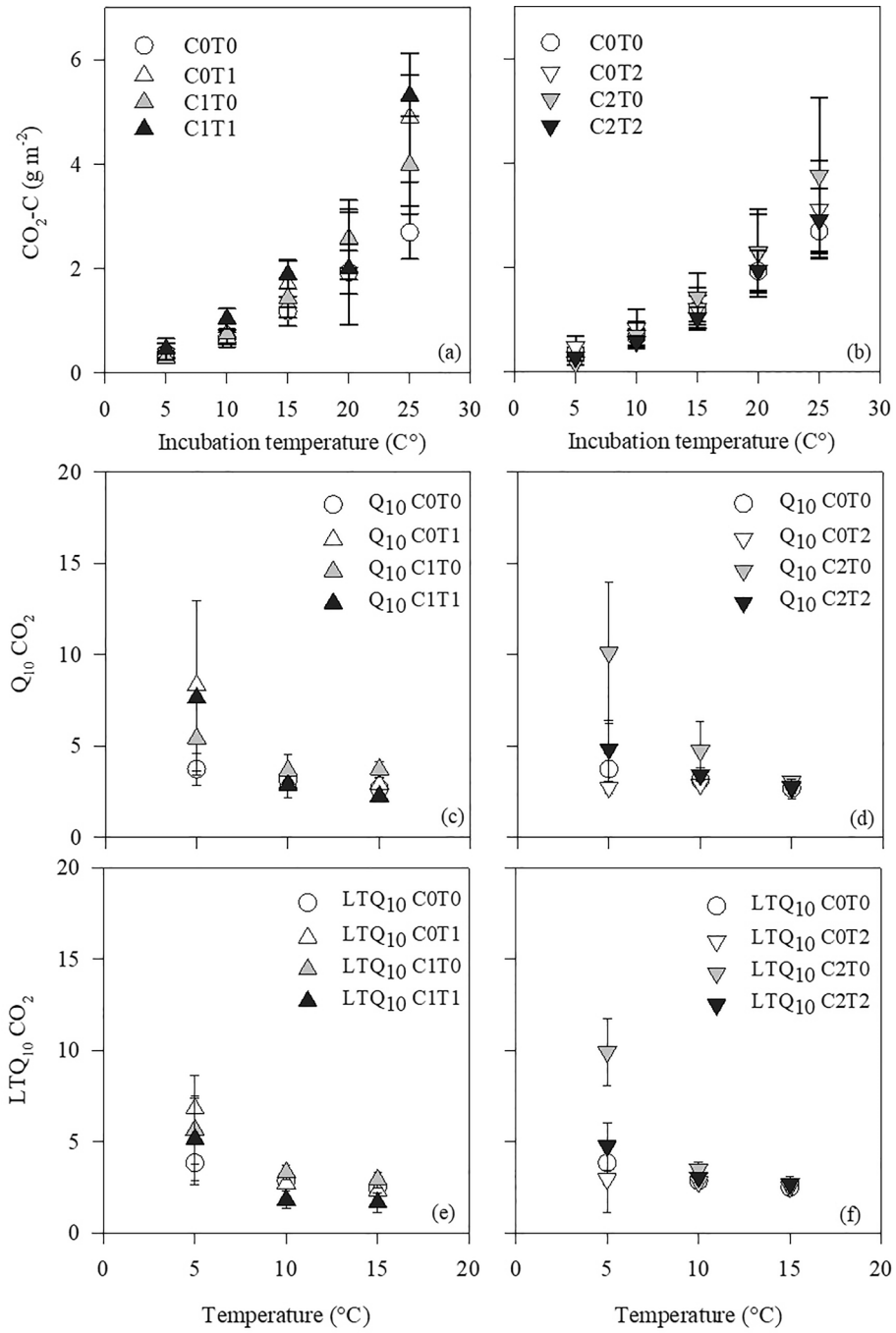

Fig. 3. Soil CO_2 fluxes measured at different lab incubation temperatures (a & b), the calculated temperature sensitivity (Q_{10}, c & d) and the temperature sensitivity modeled according to Lloyd & Taylor (LTQ_{10}, e & f). Extreme treatments (inverted triangles) on the right (b, d & f) and moderate treatments (triangles) on the left (a, c & e) with respect to the control (circles). Symbols indicate the mean values, and error bars show the standard error.

2012; Flanagan et al., 2013), but this effect will likely decline with sustained soil warming until it has again reached soil CO_2 emissions similar to control plots (Romero-Olivares et al., 2017). At an even-aged, mixed deciduous forest with buried heating cables, the respiration measured in the field was consistently higher during a seven-year treatment period (Melillo et al., 2011). In our measurements, we could see the effects of the field treatment even in the lab incubations, which hints towards a legacy effect even after two years of treatment.

Moderate eCO_2 treatments showed stronger effects on CO_2 emissions than their extreme counterpart (Fig. 1 and Table 2), which indicates a non-linear impact of eCO_2 on CO_2 emissions. Dusenge et al. (2018) suggest that eCO_2 has led to large stimulations in photosynthesis since the Industrial Revolution, but that future eCO_2 may have less dramatic effects on plant carbon uptake. This, in turn, may lead to less substrate availability for soil microbial respiration and might support the decrease of CO_2 emissions at extreme CO_2 concentrations similar to our C2T0 treatment. We suggest that the rate of soil respiration change also strongly depends on the level of eT, eCO_2 and plant composition, which might be determining for soil CO_2 emissions. Plots from our experimental field are characterized by a substantial variation in aboveground plant composition, as is typically found in grassland ecosystems. This positively affects soil trace gas emissions. Alterations in root biomass from different soil cores have been taken into account for data evaluation, but plant composition was not recorded. Root

Table 4
Two-way ANOVA for CO_2 and N_2O; eCO_2 and eT were fitted as qualitative factors with the two main effects and a two-way interaction accounted for repeated measures (incubation Temperature = Inc T; 5, 10, 15, 20, 25 °C).

	CO_2			N_2O		
Source	*df*	*F*-value	*p*-value	*df*	*F*-value	*p*-value
eCO_2	2	0.53	0.5879	2	0.83	0.4380
eT	2	0.47	0.6262	2	3.97	***0.0214***
eCO_2 × eT	2	0.80	0.4508	2	1.85	0.1621
Inc T	4	39.48	< ***0.0001***	4	54.16	< ***0.0001***
eCO_2 × Inc T	8	0.59	0.7849	8	0.72	0.6767
eT × Inc T	8	2.53	***0.0138***	8	0.79	0.6150
eCO_2 × eT × Inc T	8	1.92	0.0618	8	0.30	0.9658

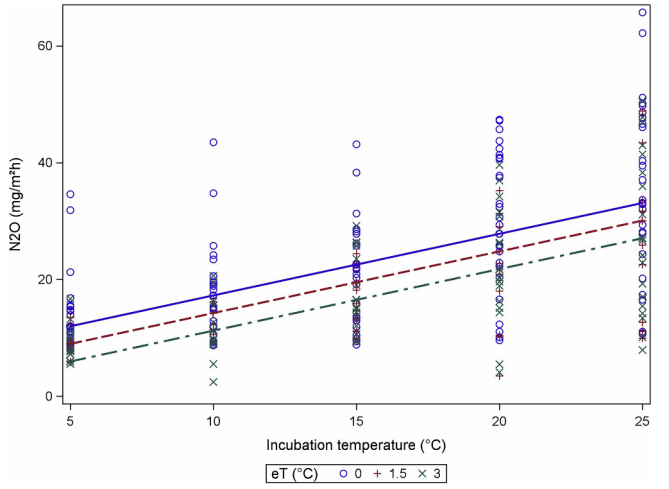

Fig. 4. Linear regression on temperature for N_2O fluxes at different incubation temperatures. The soil (blue) line and circles represent control treatment, dashed (red) line and "+" represent 1.5 °C and dotdashed (green) line and "×" represent 3 °C at increasing incubation temperature. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

biomass correlated positively with $cumCO_2$ emissions (Table 3). The latter suggests that roots might have substantially contributed to soil CO_2 emissions, most likely through a combination of increased autotrophic respiration, promotion of microbial respiration by root exudates and stimulation of decomposition rates. Moreover, $cumCO_2$ emissions revealed a positive correlation with NH_4^+ (Table 3), indicating a relation between soil respiration and microbial mineralization of C and N.

4.2. Soil N_2O emissions

Interestingly, all climate change treatments tended to decrease mean N_2O emissions concerning C0T0. Extreme eCO_2 treatment (C2T0) showed the highest, and eT treatments (C0T1 and C0T2) revealed the lowest N_2O emissions compared to other climate change treatments (Table 2).

Arnone and Bohlen (1998) found positive effects of eCO_2 on N_2O emissions from grassland monoliths already after two growing seasons, which was mainly associated with improved soil water conditions. Elevated CO_2 indirectly influences water regimes of plants, due to reduced stomatal aperture and hence decreased transpiration (Morgan et al., 2004; Morgan et al., 2011). These processes may take years to change (Morgan et al., 2004) and strongly depend on the individual plant species (Zak et al., 2000). A minimum of four years of eCO_2 was found to significantly stimulate N_2O emissions (Kammann et al., 2007). In many cases, however, these positive effects on soil water appear seasonally and mainly when water is limited (Morgan et al., 2004; Roy et al., 2016; Selsted et al., 2012). Therefore, enhanced soil water conditions as a response to eCO_2 were mostly found in water restricted ecosystems, such as semiarid grasslands (Dijkstra et al., 2010; Nelson et al., 2004).

Warming generally causes stronger impacts on soil moisture than eCO_2 (Dermody et al., 2007). Soil drying occurs as a direct effect of warming, due to higher evapotranspiration rates (Liu et al., 2009). In several laboratory incubation studies, a reduction in soil moisture on sites subjected to eT is reported and indicates a close relationship between soil moisture and N_2O emissions (Bateman and Baggs, 2005; Dobbie and Smith, 2001; Werner et al., 2014), similar to our study in which WFPS correlates positively with N_2O emissions (Table 3). The WFPS in our study is a proxy at the time point of field sampling, however it is representative for treatment effects over the growing season. This is depicted by data on soil moisture content recorded in biweekly intervals on the whole set of treatments in the field, data are shown in the Supplementary Material (Fig. S.2). However, current study design and methodological constraints do not allow any conclusions to be drawn on the mechanisms behind the interaction.

Hence, in our study clearer trends are to be expected with sustained climate change manipulation treatments. Probably the relatively high spatial variability found for soil N_2O emissions within each plot (two cores measured per plot) diminished treatment-related differences in N_2O emissions. High spatial heterogeneity was also reported by other studies for tropical montane forests (Arias-Navarro et al., 2017) as well as in temperate grasslands (Ambus and Christensen, 1994; Flechard et al., 2007; Harris et al., 2018). So-called "hot spots", microsites with unusually high emission rates, can be found on a narrow scale (< 1 m) and largely contribute to mean N_2O emissions. Hot spots can be developed by various factors, such as small-scale variations in plant cover and rooting densities, soil properties or nutrient availability (Butterbach-Bahl et al., 2013). The latter includes the accumulation of particulate C (Parkin, 1987), which also very likely caused considerable heterogeneity of soil N_2O emissions in our study. As already described in Section 4.1, our soil cores were covered with various plant species and different species were observed on each core. Dead plant material on the soil surface, which was present in the un-sieved soil samples, could already have contributed substantially to N_2O emissions, as has also been reported by Parkin (1987).

4.3. Soil NO_x emissions

In our study $cumNO_x$ emissions correlated positively with ETN and NO_3^- (Table 3). Pilegaard (2013) suggests the availability of N in the soil as most crucial factor for NO_x emissions. However, higher cumulative NO_x emissions ($cumNO_x$) in our study were caused by the warming treatments C0T1 and C0T2 (Table 2). In the surface response approach, $cumNO_x$ responds significantly to the single climate change factor temperature; this means $cumNO_x$ emissions increase linearly with eT (Fig. 2). We assume that the eT treatments influenced $cumNO_x$ emissions predominantly through their adverse effect on soil moisture, which is well in line with the suggestion of Schaufler et al. (2010) who assume that soil NO_x emissions will increase with warmer and drier climate conditions in large parts of Europe. The effect on the extreme eT (C0T2) was weaker, probably due to its lower microbial biomass (C_{mic}) (Table S.1). In our study, a negative correlation was found between mean N_2O and mean $cumNO_x$ emissions. This suggests that higher N_2O emissions are accompanied by lower NO_x emissions or vice versa. A significant contribution to NO_x emissions is related to nitrification, which occurs mainly in dry, well-aerated soils, i.e., when WFPS is low (Fumagalli et al., 2016; Schaufler et al., 2010). By contrast, increasing anaerobic microsites through increasing water saturation can boost N_2O emissions via denitrification (Schaufler et al., 2010). However, in wet soils, NO_x can be consumed in denitrification before it is transported to the atmosphere and would likely contribute to higher productions of N_2O (Pilegaard, 2013).

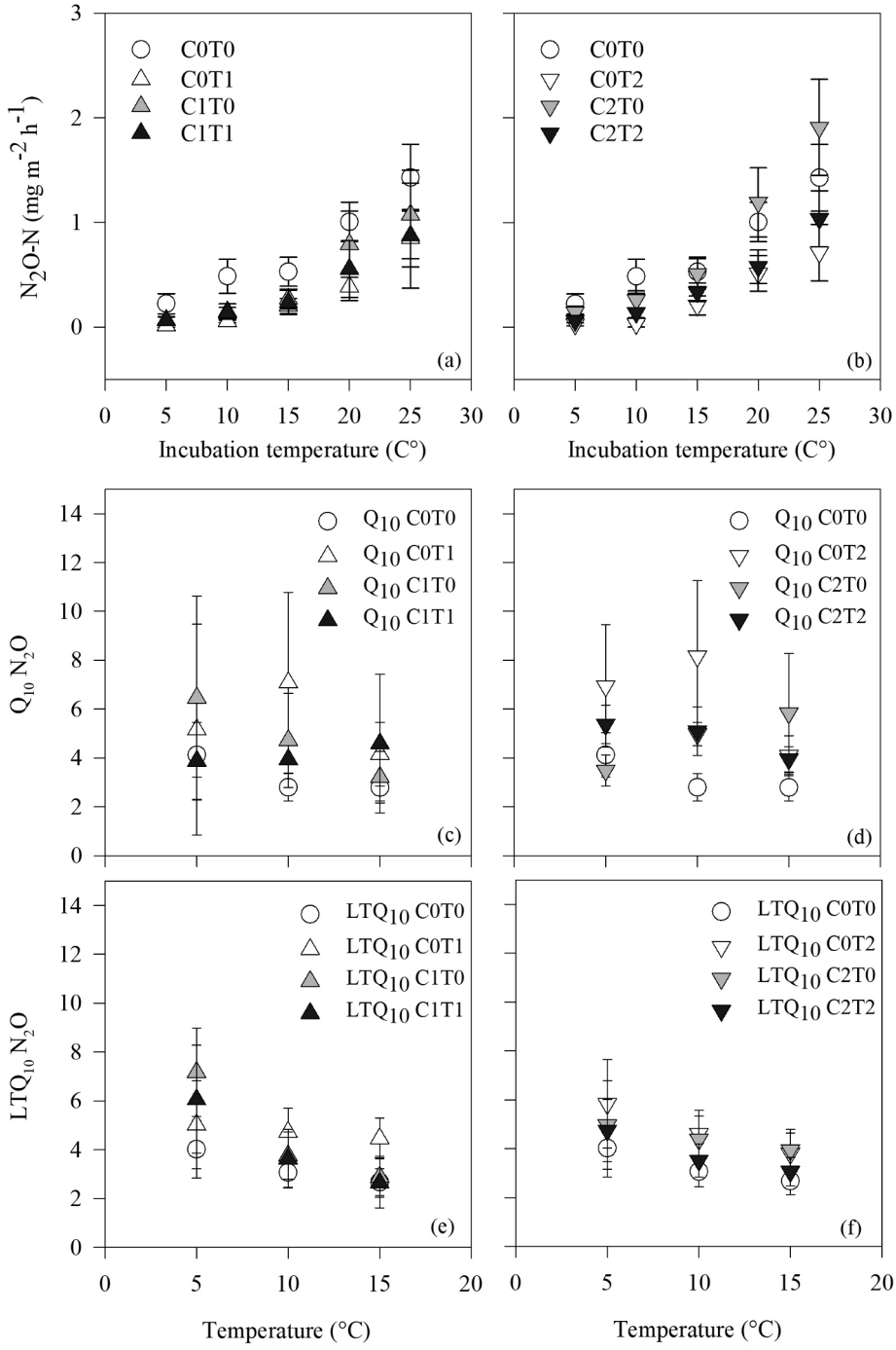

Fig. 5. Soil N_2O fluxes measured at different lab incubation temperatures (a & b), the calculated temperature sensitivity (Q_{10}, c & d) and the temperature sensitivity modeled according to Lloyd & Taylor (LTQ_{10}, e & f). Extreme treatments (inverted triangles) on the right (b, d & f) and moderate treatments (triangles) on the left (a, c & e) with respect to the control (circles). Symbols indicate the mean values, and error bars show the standard error.

4.4. Soil NH_3 emissions

To our knowledge, this study is the first to assess NH_3 emissions ($cumNH_3$) from intact soil cores in association with a climate manipulation experiment on a Central European grassland site. Surprisingly, eT treatments decreased $cumNH_3$ emissions, whereas the moderate and extreme interactions led to an increase. However, no significant differences could be observed (Table 2). The exchange of NH_3 between biosphere and atmosphere is bi-directional as soils can either act as a source or a sink. However, an increase in temperature usually favors the transformation of dissolved to gaseous NH_3, thereby generally fostering NH_3 emissions. This holds only if other main controlling factors such as NH_4^+ availability or pH-value are constant (Flechard et al., 2013). Even though a temporary increase in temperature might actively raise NH_3 emissions (Flechard et al., 2013), this does not seem to apply for two years of eT. Nevertheless, even if the direct response of NH_3 emissions to eT is diminished in the long run, indirect effects of eT, such as declining WFPS or NH_4^+ depletion, might be determining factors.

4.5. Temperature sensitivity of CO_2 and N_2O

Soil CO_2 emissions increased with incubation temperature (Fig. 3a,

b and Table 4). This is in line with previous laboratory incubation studies for different ecosystems (Fang and Moncrieff, 2001; Gritsch et al., 2015). Besides that, neither the actual Q_{10} (Fig. 3c and d) nor the LTQ_{10} (Fig. 3e and f) values of the incubated samples show clear treatment trends; we observed only a consistent increase in temperature sensitivity with decreasing temperatures. However, Q_{10} of soil respiration was not lowered by eT, as suggested by previous authors (Kirschbaum, 2004; Lloyd and Taylor, 1994; Zheng et al., 2009). There are several other environmental factors, such as substrate availability, chemical soil properties, microbial abundance and microbial structures that affect the temperature sensitivity of soil respiration (Liu et al., 2017).

Mean N_2O emissions increased with incubation temperature (Fig. 5a and 5b) as described previously (Díaz-Pinés et al., 2014). The N_2O emissions respond significantly to the main effect of temperature, which was fitted as a qualitative factor and analyzed in a two-way interaction that accounted for repeated measures (Table 4). It is known that warming suppresses N_2O emissions (Jansen-Willems et al., 2016). This is corroborated by the response of N_2O emissions to elevated air temperature in the linear model (Fig. 4). As warming did not significantly reduce mean N_2O emissions in our study (as described above in Section 3.2), we suggest that the warming effect of the field treatments only becomes visible with repeated measurements combined with increasing temperature in the laboratory.

In contrast to CO_2, actual Q_{10} of N_2O did not show a consistent increase in temperature sensitivity with decreasing temperatures (Fig. 5c and d). LTQ_{10} decreased only slightly with increasing incubation temperature (Fig. 5e and f). Soil N_2O formation involves many different soil processes and various microorganisms, which are all strongly stimulated by temporarily increased temperatures, leading to a multiplying effect of soil temperature on N cycling and N_2O emissions. Additionally, N_2O emissions are strongly dependent on soil moisture, oxygen supply, substrate availability and many other environmental factors (Butterbach-Bahl et al., 2013).

Therefore, factorial change manipulations did not affect temperature sensitivity of microbial-derived GHG after two years of field treatment.

5. Conclusion

Our results highlight the importance of considering different steps of enrichment and warming as well as combined manipulation treatments (eCO_2 and eT) in future studies to evaluate synergetic, antagonistic or additive effects since maximum CO_2 emissions occurred at the moderate eCO_2 level while extreme treatments showed similar CO_2 emissions as the control. On the contrary, the linear model in the response surface approach suggests the highest NO_x emissions occur with eT, whereas warmed and dry soils tend to lead to the lowest N_2O emissions. The latter indicates that N_2O and NO_x emissions were mainly influenced by the indirect effects of warming on soil moisture. No significant legacy effects affecting temperature sensitivity of microbial trace gas emissions were found after two years of future climate change scenarios. Clearer long-term trends are to be expected with sustained climate change manipulation treatments. Based on currently available data it is assumed that secondary effects through alterations in plant performance (e.g., stomatal closure and evapotranspiration) and abiotic soil characteristics (e.g., water content) are primary drivers for soil microbial processes, which in turn determine trace gas emissions. It is thus of the utmost importance to disentangle these complex interactions to improve predictions of climate change scenarios on biogeochemical soil processes.

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Declarations of interest: none

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2018.12.023.

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